Increasingly, mammalian miRNA are also being implicated in stress response signaling and other pathways that dynamically respond to time-varying inputs. We experimentally demonstrated temporal pulse generation in a synthetic microRNA-based IFFL and thus showed that the same miRNA can participate both in the generation of transient gene expression pulses in response to a change in an upstream regulator and in buffering steady state expression levels against such changes. We further showed that it is possible to modulate pulse shape and fine-tune steady state levels by independently controlling the relative expression levels of the miRNA, its upstream regulator, its downstream target, and the interactions between them.

2741-Symp
Gene Expression Genomics
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Gene expression levels are subject to gross regulation as well as fine-tuning. Internal and external sources of noise are superimposed on top of transcriptional control mechanisms. Data mining of transcriptomic and epigenomic measurements yield insights into general principles of regulation of gene expression.
Control of expression levels is exerted through a combination of transcription factors against a background of repression by nucleosomal histones. We have analyzed to what extent the intrinsic DNA binding preferences of yeast TFs and histones play a role in determining nucleosome occupancy, in addition to nonintrinsic factors such as the enzymatic activity of chromatin remodelers (Charoensawan et al., Mol. Cell, 2012).
Transcription factor and epigenetic control in animal cells gives rise to two major expression levels, which vary by roughly one to two orders of magnitude. This gives rise to bimodal distributions of gene expression levels in cell populations (Hebenstreit et al., Mol Sys Biol., 2011). Analysis of histone modifications by ChIP-seq indicates that activating modifications such as H3K9/14ac and H3K4me3 are involved in this 'digital' expression switch (Hebenstreit et al., Nucleic Acids Res, 2011).
These findings have broad implications for the analysis RNA-seq and ChIP-seq data, and for the understanding of the regulation of gene expression in eukaryotic cells.

Platform: Imaging & Optical Microscopy - Technology

2742-Plat
Reductive Caging enables Ultra-Bright Photoactivatable Fluorophores for Superresolution Imaging
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From GFP to intracellular sensor dyes, developments in fluorescent probes have been major driving forces in imaging-based biological research. Recently, photoswitchable fluorophores have enabled super-resolution imaging based on the sequential localization of individual fluorescent molecules (STORM, PALM, etc.), such that researchers may now use widely available commercial instrumentation to study biological structures at ~20-50 nm distance scales.
We report a chemical strategy for fluorophore caging by reduction that creates photoactivatable fluorescent probes with ultrahigh photon yields. The caging process is achieved simply and rapidly by treating a labeled sample with an aqueous reducing agent to convert the fluorophores to a long-lived reduced and nonfluorescent form. Upon photoactivation, these probes can provide up to 1,700,000 detected photons per photoswitching event and allow localization precision as high as 1-2 nm. The photon yield is 2-3 orders of magnitude higher than previously available photoswitchable/photoactivable probes. The improved image resolution allows biomolecular structures previously unobservable by super-resolution fluorescence microscopy to be resolved now. The novel reductive caging and photoactivation method is broadly applicable to many fluorophores spanning the visible spectrum.

2743-Plat
Developing Photoactivatable Fluorescent Proteins for Diffraction-Limited and Superresolution Imaging
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Photoactivatable fluorescent proteins (PAFPs) are molecules that switch to a new fluorescent state in response to specific light activation, and play vital roles in super-resolution imaging. There are three classes of PAFPs: dark-to-bright photoactivators (PAFPs), irreversible photoconverters (PCFPs), and reversible highlighters (RSFP). However, compared to traditional fluorescent proteins (such as GFP or RFP), only limited PAFPs are available for super-resolution microscopy.
Previously, we developed several novel PAFPs, mGeos, with various switching rates, photon numbers and brightness1. And based on the crystal structure of green state mEos2, we evolved two truly monomeric and bright RSFPs, mEos3.1 and mEos3.2, with the good photochemical properties including rapid maturation rate, high photon budget and extremely high labeling density2. Here we present new generation of mGeos2 which are true monomeric PAFPs. These novel fluorescent proteins are suitable for both single color and dual color PALM superresolution imaging, and have a broad brand of applications in traditional fluorescence microscopy such as dynamic tracking and pulse chase labeling of proteins.
1. Chang H, Zhang M, Ji W, Chen J, Zhang Y, Liu B, Lu J, Zhang J, Xu P, Xu T, A new series of reversibly switchable fluorescent proteins with beneficial properties for various applications, Proc Natl Acad Sci U S A. 2012 Mar 20;109(12):4455-60.
2. Zhang M, Chang H, Zhang Y, Yu J, Wu L, Ji W, Chen J, Liu B, Lu J, Liu Y, Zhang J, Xu P, Xu T. Rational design of true monomeric and bright photoactivatable fluorescent proteins. Nat Methods. 2012 May 13;9(7):727-9.

2744-Plat
Resolft Nanoscopy in Life Sciences: Unraveling Fine Details with Low Light Levels
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Lens-based fluorescence microscopy, which has long been limited in resolution to $>$ 200 nanometer by diffraction, is rapidly evolving into a nanoscale imaging technique. Here, we show that emergent RESOLFT fluorescence microscopy enables fast and continuous imaging of sensitive, nanosized features in living brain tissue. using low intensity illumination to switch photochromic fluorescent proteins reversibly between a fluorescent ON-state and a non-fluorescent OFF-state, we obtained more than a 3-fold increase in all three spatial dimensions over that of confocal microscopy. Dendritic spines located 10-50 μm deep inside living organotypic hippocampal brain slices were recorded for hours without signs of degradation. using a fast-switching fluorescent protein increased the imaging speed 50-fold over reported RESOLFT schemes, which in turn enabled us to record spontaneous and stimulated changes of dendritic actin filaments and spine morphology occurring on time scales from seconds to hours.

2745-Plat
SW 2PE-STED Nanoscopy
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With this work we present a new approach to two-photon excitation - stimulated emission depletion microscopy (2PE-STED), exploiting the very same wavelength for excitation and depletion [1]. It is well known that two-photon excitation (2PE) fluorescence microscopy is a technique particularly suitable for three-dimensional (3D), deep tissue and in vivo imaging applications. Since 2009, 2PE microscopy has been proposed coupled with stimulated emission depletion (STED) technique, bringing the super-resolution ability to the multi-photon excitation technique [2, 3].
Unfortunately, the use of two distinct wavelengths for excitation and depletion requires mostly a special optical filter design to make the setup invariable in terms of the choice of the marker dye and potential light beam distortions have to be treated separately. Working with only just one wavelength for excitation and STED one would directly simplify the imaging formation scheme. We propose an imaging method to perform 2PE-STED imaging using a single wavelength (SW) and, consequently, the very same laser source for 2P excitation and depletion. We show that this method allows super-resolved imaging using a standard fluorophore like ATTO647n achieving a resolution below 80nm. Therefore it allows an easy coupling to a conventional commercial confocal microscope. The SW 2PE-STED nanoscopy is a promising technique to better actively control distortions when imaging thick highly scattering specimens, it will allow foreseeing advances in the imaging of thick specimens at nanoscale resolution.
[1] P. Bianchini, B. Harke, S. Galiani, G. Vicidomini, and A. Diaspro, ''Single-wavelength two-photon excitation-stimulated emission depletion (SW 2PE-STED) superresolution imaging'', PNAS 109, 6390-6393 (2012).
[2] G. Moneron and S. W. Hell, ''Two-photon excitation STED microscopy,'' Opt Express 17, 14567-14573 (2009).